

RMRC Genotyping Protocol-PCR

No.	RMRC13273 RMRC13274	Strain Name	NOD-Tg(Lck-cre)7Sytwu NOD-Tg(Lck-cre)14Sytwu
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A. Primer

	Primer name	Sequence
1	P06-39	TAA TGA AGA GGG ACA GGT ACC CTC
2	P07-04	TGC ACG TTC ACC GGC ATC AAC GTT

B. Reaction Conditions

Component	Concentration		Volume		Step	Temperature, °C	Time, sec	Number of Cycles
				μl				
H ₂ O	--		12.2	μl	1	94	300	1
PCR buffer	10x		2	μl	2	94	30	30
dNTP	2.5	mM	4	μl	3	57	60	
Primer 1	20	μM(μmol/μl)	0.25	μl	4	72	45	
Primer 2	20	μM(μmol/μl)	0.25	μl	5	72	420	1
DNA	(100 ng/μl)		0.5	μl	6	25	120	1
Taq	5	U/μl	0.2	μl	7			
Mg ²⁺	50	mM	0.6	μl	8			
Total			20	μl	9			

C. PCR Product

	Primer set		Product size	Target region
1	P06-39	P07-04	719	From <i>Lck</i> exon 1 to transgenic <i>Cre</i> (396-419bp)

D. Gel Photo

